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DRUG SENSITIVITY OF MICROBIAL AGENTS ISOLATED FROM COWS WITH SUBCLINICAL AND CLINICAL MASTITIS TO SOME ANTIBIOTICS

G. BOZHKOVA[1], P. ANDREEVA[2] AND Y. PETKOV[3]

[1] Post-Graduate Training Centre, Sofia; [2]Antibiotik Ltd., Razgrad; [3]Faculty of Veterinary Medicine, Trakia University, Stara Zagora; Bulgaria

Summary

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Pathogenic microbial strains, isolated from cows with clinical and subclinical mastitis were shown to have a various sensitivity to some antibiotics.

The combined *in vitro* application of gentamicin and lincomycin resulted in relatively low fractional inhibitory concentration (FIC) indexes in a part of *Staphylococcus aureus* and *Streptococcus agalactiae* strains, evidencing the presence of a synergic effect.

S. aureus, Streptococcus epidermidis, S. dysgalactiae, S. uberis and *Escherichia coli* were sensitive to gentamicin (90.4-100%), streptomycin (66.6-100%), lincomycin (100%; 33.3% for *E.coli*), ampicillin (33.3-100%); penicillin (16.1-100%). *S. agalactiae* and *Actinomyces pyogenes* strains were shown to be fully sensitive to gentamicin and lincomycin (100%).

Key words: antibiotics, cows, drug interaction, mastitis, minimum inhibitory concentrations, synergy

INTRODUCTION

The long-term treatment of clinical and subclinical mastitis in ruminants with the same antibiotics always results in increase in the resistance of most pathogenic microorganisms. This resistance varies with respect to the different antibiotics (Costa et al., 1985; Chandro et al., 1989; Malinowski et al., 1992).

The appearance of resistance in microbial agents, causing mastitis, depends greatly on the duration of treatment and the schedule of application of antibiotics - solely or in combination (Prasad and Khahra, 1986; Srm et al., 1989). At the same time, it is known that the simultaneous application of two antibiotics could result in enhanced or decreased antibacterial effect depending on the individual cases (Staneva-Stoycheva and Stoychev, 1984).

The aim of the present study was to determine the sensitivity of some pathogenic microorganisms causing clinical and subclinical mastitis to some antibiotics with regard to their independent or combined intramammary use.

MATERIALS AND METHODS

The study was performed with 64 bacterial strains - 54 isolated from cows and sheep with subclinical and clinical mastitis and 10 - from human patients with upper respiratory tract diseases.

The tests were performed on solid culture medium according to Muller-Hinton. The minimum inhibitory concentrations (MIC) of lincomycin (Lm) and gentamicin (Gm) against studied strains were determined. Solutions with doubly decreasing concentrations of both antibiotics were combined in various ratios. Two ml volumes of those combinations were added to 18 ml agar, melted down and cooled to 55-60 ^oC. The tests were made in Petri dishes. The inoculation of the culture medium with antbitic solutions was done using the Steers replicator. Bacterial suspensions with a density of 10^7 microbial cells/ml were used in an amount of 0.001 ml. The growth was detected following incubation at 37 ^oC for 18-20 h.

The results were analyzed on the basis of MIC of Lm and Gm after single and combined use and the fractional inhibitory concentrations (FIC) indexes, calculated as followed:

$$\text{FIC index} = \frac{MIC_{Lm+Gm}}{MIC_{Lm}} + \frac{MIC_{Gm+Lm}}{MIC_{Gm}}$$

where: MIC_{Lm} and MIC_{Gm} are MIC of lincomycin and gentamicin respectively; MIC_{Lm+Gm} is MIC of lincomycin acting in the presence of gentamicin; MIC_{Gm+Lm} is MIC of gentamicin acting in the presence of lincomycin.

The effect of the combined use was assessed as synergic in FIC index < 0.5; additive - in FIC index between 0.5 and 0.75; neutral - in FIC index between 1 and 2 and antagonistic - in FIC index > 2.

The sensitivity of 129 strains isolated from cows with clinical and subclinical mastitis to gentamicin, lincomycin, streptomycin, penicillin and ampicillin was routinely determined on a solid culture medium.

RESULTS

Table 1 presents the sensitivity of strains isolated from cows with subclinical and clinical mastitis and strains from human patients to lincomycin and gentamicin, applied solely or in combination. Most *Staphylococcus aureus* strains were sensitive to both antibiotics. The MIC of gentamicin was lower than that of lincomycin. MIC of both antibiotics against *S. aureus* strains of human origin were lower compared to those against strains of bovine origin. The *Streptococcus agalactiae* and *Streptococcus uberis* strains were sensitive to lincomycin and gentamicin but the respective MIC were significantly higher compared to those against *S. aureus*. The

Table 1. Minimum inhibitory concentrations (MIC) of gentamicin and lincomycin applied solely to various strains and FIC indexes in combined application (mean ± SEM)

Mictrobial strains	Number of strains	Lincomycin MIC (μg/l)	Gentamicin MIC (μg/l)	Lincomycin and gentamicin, (FIC index)
Staphylococcus aureus	29	0.93 ± 0.17	0.52 ± 0.19	0.87 ± 0.17
Streptococcus agalactiae	11	13.10 ± 0.17	6.63 ± 3.16	0.95 ± 0.31
Streptococcus uberis	5	4.90 ± 1.13	2.40 ± 1.41	1.20 ± 0.56
Escherichia coli	9	1024.00 ± 0.22	0.25 ± 0.08	-
Staphylococcus aureus (human isolate)	10	0.67 ± 0.22	0.12 ± 0.03	0.51 ± 0.16

Table 2. Percentage of the *in vitro* sensitivity of bacterial strains, isolated from the milk of cows with clinical and subclinical mastitis to some antibiotics

Mictrobial strains	Number of isolated strains	Sensitivity to antibiotics, %				
		Strepto-mycin	Genta-micin	Ampicil-lin	Penicil-lin	Linco-mycin
Staphylococcus aureus	68	83.8	100.0	83.8	16.1	100.0
Streptococcus epidermidis	5	100.0	100.0	80.0	80.0	100.0
Streptococcus agalactiae	20	-	100.0	-	-	100.0
Streptococcus dysgalactiae	3	66.6	100.0	100.0	100.0	100.0
Streptococcus uberis	21	100.0	90.4	33.3	100.0	100.0
Actinomyces pyogenes	9	-	100.0	-	-	100.0
Escherichia coli	3	100.0	100.0	33.3	33.3	33.3

MIC of gentamicin to *Escherichia coli* strains was comparatively lower but on the other side, *E. coli* were lincomycin-resistant.

The combined use of gentamicin and lincomycin resulted in comparatively low average FIC indexes. The analysis of data for individual strains evidenced the presence of a synergic or additive effect. The effect was synergic in 41.3% of all 29 *S. aureus* strains, isolated from cows with clinical or subclinical mastitis while in the rest - additive or neutral. A synergic effect was observed in 54.5% of *S. agalactiae* strains and additive or neutral in the remaining part. The same tendency was present in *S. uberis* strains. The effect of the combined use of both antibiotics to *E. coli* was not determined because all tested strains were highly sensitive to gentamicin and resistant to lincomycin. A synergic effect was observed in almost all *S. aureus* strains of human origin. After the combined use of gentamicin and lincomycin the synergic effect was present in a relatively broad range of ratios between both antibiotics: 31.1% in a ratio of 2:1, followed by 1:1 and rarely in rations 1:4; 4:1 and 8:1.

The determination of the percentage of sensitivity of 129 strains isolated from cows with clnical and subclinical mastitis showed a certain difference among individual strains. Almost all strains were gentamicin-sensitive. The sensitivity to streptomycin and lincomycin was high as well. Individual *Streptococcus dysgalactiae* strains were streptomycin-resistant and some *E. coli* strains - lincomycin-resistant. The predominant number of microbial strains were resistant to penicillin and ampicillin (Table 2).

DISCUSSION

The results of our experiments showed that the sensitivity of strains isolated from cows with subclinical and clinical mastitis to lincomycin and gentamicin varied within a broad range. This statement is further supported by the difference in the MIC of both antibiotics against individual strains, the sensitivity of the majority of studied strains and the resistance of *E. coli* to lincomycin. This variable sensitivity corresponds in a certain extent to data of other authors (Zurzul et al., 1991). Our results are different from those of Prasad and Khahra (1986) about the resistance of *E. coli* and *S. aureus* to gentamicin.

The relatively low values of FIC indexes following the combined application of lincomycin and gentamicin against strains causing clinical and subclinical mastitis evidence the presence of a synergic or additive effect. This is important for creating effective intramammary preparations for treatment of subclinical or clinical mastitis. The application of antibiotic combinations is purposeful because of the exceptional variety of bacterial pathogens' sensitivity and the rapid development of drug resistance. A confirmation for the possibility of higher efficacy of mastitis treatment with gentamicin, combined with another antimicrobial drug (nifuroxaside) are the respective good results obtained by Filipovic-Panincic et al. (1989).

The high sensitivity of Gram-positive and Gram-negative microorganisms to streptomycin, ampicillin and penicillin are indicative for the lack of drug resistance in our cases although their continuous use in the veterinary practice. Similar data are reported by Ziv (1995) and Pege et al. (1990). Usually, streptococci are sensitive to many antibiotics. Some staphylococci are however resistant to some penicillin antibiotics (Orm et al., 1989). A significantly higher resistance of mastitis agents is reported by Costa et al. (1985).

CONCLUSIONS

The combined *in vitro* application of gentamicin and lincomycin resulted in relatively lower FIC indexes in part of *Staphylococcus aureus* and *Streptococcus agalactiae* strains, evidencing the presence of a synergic effect. The most suitable combination of lincomycin and gentamicin was in a ratio of 2:1 or 1:1.

The strains isolated from cows with clinical or subclinical mastitis were sensitive to gentamicin, streptomycin and lincomycin excepting most of *E. coli* strains that were resistant to lincomycin.

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Correspondence:
Res. Scientist Dr. G. Bozhkova
Post-Graduate Training Centre,
Shosse Bankya str. 9,
1331 Sofia, Bulgaria